

CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

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Renewal
Application for Research Grant

#415R1

Activated: 1/1/65

cf. #155

7/1/57 - 9/1/59

cf. #345

9/1/62 - 2/28/64

Date: February 1, 1966

1. Name of Investigator: DUANE G. WENZEL, PH.D.

2. Title: Professor of Pharmacology

3. Institution &
Address: The University of Kansas
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Lawrence, Kansas 66044

4. Project or Subject: ANTIHYPERTENSIVE ACTIVITY OF NICOTINE

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

Introduction: Work from our laboratories has been reported which demonstrates that the chronic oral administration of nicotine to rats first elevates the systolic blood pressure and then reduces it to the original level or below. Further, renal hypertension was reduced to normal by this treatment. Since the above study was reported, the project supported by the Tobacco Research Council has made the following general findings to date: (more detailed evidence in attached progress report)

- (1) Increasing the dose of nicotine from 1.14 or 2.28 mg/kg/day to 4.56 mg/kg/day eliminates the initial pressor response.
- (2) This larger dose administered concurrently with the production of renal hypertension permits the development of hypertension, although the pressor response peaks and falls faster than when a lower dose of nicotine was employed in the previous study (3 weeks vs. 15 weeks).
- (3) Beginning administration of the larger dose of nicotine to rats with fully developed renal hypertension produces a rapid fall in blood pressure (30 mm Hg in three weeks).

1. Wenzel, D. G., Wattanapongsiri, A. and Vedral, D., Nicotine and Renal Hypertension in the Rat, J. Pharmacol., 145, 315 (1964).

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5. Detailed Plan of Procedure (continued)

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- (4) Rats treated with 4.56 mg/kg/day of nicotine for 14 weeks demonstrate increased sensitivity to the pressor effect of norepinephrine; a reduced response to tyramine; a slight but significant sensitization to vasopressin; and no change in the response to angiotensin.

On the basis of the above results and the known effects of nicotine on catecholamine stores, the following tentative hypothesis is offered as an explanation: The pressor effect of nicotine is related in large measure to the release of norepinephrine stores while the depressor effect results from the depletion of these stores.

This hypothesis will be directly and indirectly tested by a combination of two methods: (1) measurement of the contractile response of norepinephrine-dependant isolated smooth muscle from rats chronically treated with nicotine, and (2) measurement of epinephrine and norepinephrine stores in this muscle as well as in other tissues from the same animals.

Basis for Use of Vas Referens: Initial investigations have been underway since the summer of 1965 to determine the suitability of the rat hypogastric nerve-vas deferens preparation for the study of the chronic effects of nicotine. An improved apparatus has been designed to measure the isotonic contractions of the vas deferens when stimulated alternately through the muscle itself and through the hypogastric nerve. Details of the effects of varying stimulus parameters are still under study.

The isolated hypogastric-vas deferens preparation is being employed because of its unique characteristics. It is one of the few satisfactory isolated nerve-smooth muscle preparations currently known having autonomic ganglia, post-ganglionic adrenergic fibers, and pre- and post-ganglionic cholinergic fibers. Further, the effects of chronic nicotine treatment at these sites may be determined through the use of different electrical stimulus parameters and the use of pharmacological agents added in vitro i.e. Low frequencies (5-10 cps) through the hypogastric nerve release acetylcholine at the end organ while high frequencies (25-50 cps) release norepinephrine. High frequencies, applied directly to the muscle, stimulate postganglionic adrenergic fibers while low frequencies of long duration directly stimulate the smooth muscle cell.

Experimental Design: Two separate groups of male Sprague-Dawley rats will be administered 1.14 or 4.46 mg/kg/day of nicotine in the drinking water. Eight rats will be sacrificed from each group on days 1, 7, 21, 42 and 84. A similar untreated control group will be used with eight animals for each period of testing. The mean contractile force of the electrically-stimulated vas deferens-hypogastric nerve preparations will be determined at frequencies of 1, 5, 15, 30 and 50 cps applied alternately to nerve and directly to the muscle. Pulse duration is to be 2 milliseconds, period of stimulation 5 seconds, and stimulus amplitude supramaximal (20 volts nerve and 60 volts muscle). Once the frequency-response series is completed for both nerve and muscle on a given preparation, it will be treated in vitro with atropine (1×10^{-6}) with responses determined at 5 and 50 cycles, and next with phentolamine (0.1×10^{-6}) using the same test procedure.

Further evaluation of the effects of chronic nicotine upon the vas deferens will depend upon initial results. It is anticipated that it will be both desirable and necessary to essentially duplicate the above experiment with the substitution of acetylcholine or norepinephrine for electrical stimulation.

Part II. Concurrently with the above procedure, specific organs will be isolated from each test animal and will be analyzed for their epinephrine and norepinephrine content by spectrofluorometry. The organs will include: the remaining vas deferens, both adrenals, heart, and aorta.

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6. Budget Plan:

a. Salaries	\$ 8,280
b. Expendable Supplies	1,200
c. Permanent Equipment	750
d. Overhead (15% of a, b, c)	1,452
e. Other	200
Total	\$11,882

7. Anticipated Duration of Work: The study, as outlined, should take about one year. (See 10)

8. Facilities and Staff Available: The School of Pharmacy has six pharmacology research laboratories. One or more of these would be devoted exclusively to continuing the research relative to the effect of nicotine on the blood pressure. A modern, fully-staffed animal room is used for such research programs. A Science Library holding over 60,000 volumes occupies one floor of our building.

The School of Pharmacy has four pharmacologists on its staff as well as four medicinal chemists and specialists in other areas who may serve as consultants.

9. Additional Requirements: None

10. Additional Information (including relation of work to other projects and other sources of supply): (From 7)

If the results of this year's study corroborate the evidence that nicotine is acting to produce a chemical sympathectomy, further work will be desirable in order to (1) make a comparison of the blood pressure effects and side-effects of chronic nicotine and syrosingopine treatments; (2) determine the effect of chronic nicotine upon rats having genetic and salt-DOCA hypertension; (3) determine the systolic and diastolic b.p. response of chronic nicotine-treated rats subjected to drugs (eg. pressor agents) and treatments (eg. carotid occlusion) via arterial cannulation.

Signature

Dwaine H. Wenzel

Director of Project

William J. Argersinger, Jr.

Business Officer of the Institution

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